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Toxicity and Phototoxicity of Mixtures of Highly Lipophilic PAH Compounds in Marine Sediment: Can the ΣPAH Model Be Extrapolated?

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Abstract. The additivity of toxic units was tested using sediments contaminated with mixtures of highly lipophilic (log $K_{ow} > 4.5$) parent and alkylated PAHs. The direct toxicity and photoinduced toxicity of these mixtures were examined in standard 10-day sediment toxicity tests using the infaunal amphipod *Rhepoxinius abronius*, with mortality and the survivors' ability to rebury as endpoints. Survivors of the initial 10-day tests were then exposed for 1 h to ultraviolet (UV) radiation and the results compared to initial (10-day) endpoints. Tissue residues and lipids were measured and biota-sediment accumulation factor (BSAF) values determined. The results indicated that the bioaccumulated contaminants were not initially toxic, however, they were highly phototoxic. Although the summed toxic units of these contaminants appeared to be nonadditive, additivity was not disproved as inaccuracies in extrapolating the K_{ow}-LC50 QSAR or insufficient exposure duration might also have accounted for the observed results. Critical body residue (CBR) estimates for *R. abronius* were similar while BSAF values were much larger (10×) in comparison to other studies, which used amphipods and PAHs. The phototoxicity of mixtures of contaminants were similar to the phototoxicity of single contaminants when expressed on a molar basis, which suggests that phototoxicities may be roughly additive.

The ΣPAH model was developed at our laboratory to predict the 10-day cumulative toxicity of sediment mixtures of polynuclear aromatic hydrocarbons (PAHs) to marine amphipods (Swartz *et al.* 1995). An assumption of this model is that the cumulative toxicity of a sediment may be estimated by summing the toxic unit (TU) values of each PAH, where 1.0 TU = LC50 concentration. Additional work at our laboratory (Swartz *et al.* 1997) using sediments contaminated with equitoxic mixtures of acenapthene, phenanthrene, fluoranthene, and pyrene supported the assumption of TU additivity. However, further research was needed to evaluate the TU additivity of mixtures of contaminants that exhibited little or no 10-day toxicity when amphipods were exposed in single compound experiments. Thus, a goal of the present research was to further test additivity using standard 10-day sediment toxicity tests (ASTM 1996a). This was accomplished by exposing amphipods to mixtures of highly lipophilic parent and alkylated PAH contaminants, each of which have been shown in previous 10-day tests to be nontoxic (Boese *et al.* 1998).

Another goal was to determine the critical body residue (CBR) for neutral narcotic contaminants in our standard marine amphipod test organism, *Rhepoxinius abronius*. The CBR as defined by McCarty *et al.* (1992) is the tissue concentration (expressed on a molar basis) of a contaminant or combination of contaminants that causes a toxic effect (50% mortality in the present study).

In addition to their direct mode of toxic action, some PAHs exhibit up to an order-of-magnitude increase in toxicity in the presence of UV radiation (Newsted and Giesy 1987; Ankley *et al.* 1996; Swartz *et al.* 1997; Boese *et al.* 1998). This toxicity is most likely caused by the transfer of energy from the UV radiation–excited state of the contaminant to molecular oxygen, resulting in the formation of highly toxic superoxide radicals (Newsted and Giesy 1987), a reaction that occurs on bioaccumulated contaminants within the tissues of the UV-irradiated organism (Ankley *et al.* 1997). In previous work at our laboratory, photoinduced toxicity was observed in *R. abronius* exposed in 10-day sediment toxicity tests to PAHs, then removed from the contaminated sediment and exposed to UV radiation for 1 h (Swartz *et al.* 1997; Boese *et al.* 1998). Using a QSAR model (Mekenyan *et al.* 1994; Veith *et al.* 1995) we were generally able to predict which contaminants would exhibit photoinduced toxicity; however, determining which contaminants were most effective in evoking a phototoxic response (Boese *et al.* 1998) and determining if the phototoxic effects of PAH mixtures were additive (Swartz *et al.* 1997), were both hampered by the lack of tissue residue measurements. Thus, our additional goals were to relate measured tissue residues to phototoxic effects and to determine if phototoxic effects were additive.

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Materials and Methods

Sediments

Uncontaminated sediment was collected from McKinney Slough (Waldport, OR). The sediment, a muddy sand, was sieved (0.5 mm) into 28‰ seawater and allowed to settle for 24 h, at which time the overlying water was decanted and the organic carbon (OC) content of the sediment determined by combustion (Perkin Elmer 2400 CHN Elemental Analyzer, Norwalk, CT) after carbonate removal by acidification (Plumb 1981).

Contaminants were parent and alkylated PAHs that differed in octanol/water partition coefficient (K_{ow}) values and in their ability to induce phototoxic responses in the test species *R. abronius* (Table 1). Test sediments were prepared that contained single contaminants and contaminant mixtures (Table 2). Single-contaminant exposures (Table 2, treatment groups A and B) were prepared such that the interstitial water (IW) concentration of the sediment was at or near the aqueous solubility limit value for that compound (fluoranthene excepted). As published solubility limit values for highly lipophilic compounds are highly variable (Mackay *et al.* 1992), the IW solubility limit values used in the present study were empirically determined from previous IW water measurements (R. J. Ozretich unpublished data) of these same contaminants in single compound toxicity tests conducted using similar sediments, temperatures, and salinities (Swartz *et al.* 1997; Boese *et al.* 1998). Thus, the highest nominal IW concentrations listed in Table 2 (fluoranthene excluded) represent an estimate of the maximum concentration of each contaminant that could be dissolved in IW under our experimental conditions.

Treatment group C consisted of dual-contaminant exposures, each of which consisted of fluoranthene (50 nmol/L) plus one of the six contaminants from treatment group B. Treatment group D consisted of two mixtures of the six contaminants. One of these mixtures was prepared such that each contaminant was at or near its empirical aqueous solubility limit, and the other mixture was prepared such that each contaminant was at approximately 25% of this saturation value (Table 2). Treatment group E also consisted of two mixtures that were similarly prepared with the addition of fluoranthene (50 nmol/L) to each. The addition of fluoranthene, a contaminant that has been shown to have direct and photoinduced toxicity to *R. abronius* (Boese *et al.* 1997; Swartz *et al.* 1997), was done to enhance our ability to discern the additive toxicities of additional contaminants.

Contaminants were added to sediments following the method of Ditsworth *et al.* (1990). Briefly, the OC content of the sediment was determined and the amount of contaminant needed to attain the desired IW concentration calculated using an equilibrium partition model (Könemann 1981; Swartz *et al.* 1995). The contaminant was then dissolved in acetone, applied to the walls of a 2,000-ml glass rolling mill jar (typically in 5- to 10-ml volumes), and the solvent evaporated. Sediment was added to the jar, the contents mixed by rolling for 2 h (15°C), stored overnight (4°C), then rerolled for an additional 2 h (15°C). Contaminated sediments were then stored in these rolling mill jars for at least 28 days (4°C) before test initiation. Carrier control sediment (acetone without contaminant) was processed in the same manner. Negative control sediment (no solvent or contaminant) and the sediment used for reburial tests, a fine sand (OC ≈ 0.5%), was collected from the *R. abronius* collection site in Yaquina Bay (Newport, OR).

Toxicity Tests

Standard, 10-day sediment bioassays (ASTM 1996a) were performed using *R. abronius* exposed to treatment (Table 2) and control sediments. Each sediment replicate consisted of a 1,000-ml glass beaker containing 2 cm of test or control sediment, which was covered with 775 ml of seawater (28‰). Six replicate beakers were prepared of each control, single-contaminant, and multiple-contaminant sediment treatments. Each replicate beaker was given a randomly assigned number, placed overnight in a water bath (15°C) with gentle aeration (via 1-ml glass pipet), and covered with a watch glass.

Table 1. Log K_{ow} and phototoxicity potential to *Rhepoxinius abronius* of the PAHs and alkylated PAHs

Contaminant	Log K_{ow}[a]	Phototoxic?
Anthracene	4.54	Yes[b]
Benz(a)anthracene	5.91	Yes[c]
2-Methylanthracene	5.15	Yes[c]
Benzo(b)fluoranthene	5.80	Yes[c]
Chrysene	5.86	No[b]
3,6,Dimethylphenanthrene	5.30	No[c]
Fluoranthene	5.22	Yes[b]

[a] K_{ow} values from Mackay *et al.* 1992
[b] Data from Swartz *et al.* 1997
[c] Data from Boese *et al.* 1998

A maximum of 10 days before test initiation, amphipods were collected subtidally with a small biological dredge from Yaquina Bay (Newport, OR). Collected amphipods were maintained in sediment from the collection site and acclimated to bioassay salinity (28‰), temperature (15°C), and lighting conditions (continuous). At the start of each toxicity test (t_0), amphipods were sieved from the sediment then added to five of the replicate test and control sediment beakers (30 amphipods to each beaker). After amphipod additions, beakers were returned to the water bath, covered with a watch glass, and aeration resumed. The remaining abiotic single test and control replicates that did not receive amphipods were sampled for IW contaminant concentrations.

Positive controls were 4-day water-only tests (no added sediment) prepared at t_0. These positive controls consisted of seven 1,000-ml glass beakers containing $CdCl_2$ (5, 2.5, 1.25, 0.62, 0.31, 0.16, and 0 mg/L) dissolved in 975 ml of seawater (28‰). After addition of the $CdCl_2$ solutions, water samples (7 ml) for Cd analysis were taken and preserved with concentrated HNO_3 (0.001 ml/ml of sample) and stored at room temperature until analyzed. Positive control replicates were handled in the same manner as test and sediment control replicates with the exceptions that 20 amphipods were placed in each beaker and there was only one replicate per treatment.

During the test, visual observations were made daily with obvious mortalities and unusual conditions noted. Amphipods that had become entrapped at the air-water interface were gently tapped beneath the water surface to allow them to rebury.

At the end of the test (t_{10}), amphipods were gently sieved (0.5 mm) from the test and control sediment; survivors were counted and placed into glass culture dishes (10 cm diameter, 4 cm deep), each of which contained approximately 200 ml of negative control sediment covered by 2 cm of seawater (28‰). After 1 h, the number unable to rebury were counted. Positive controls were similarly sampled and evaluated after 4 days of exposure (t_4 of each bioassay).

UV Exposures

After the reburial tests (t_{10}), a single replicate from each treatment was randomly selected for phototoxicity testing. Survivors (including those that did not rebury) were sieved (0.5 mm) from the reburial sediment and placed into individual plastic petri dish lids (95 mm diameter, 7 mm deep) containing 30 to 50 ml of seawater (28‰, 15°C) and exposed to UV light for 1 h in a growth chamber (Model GC15-H, Environmental Growth Chambers, Chagrin Falls, OH) maintained at 15°C. Following UV exposure, mortalities and the survivors' ability to

Table 2. Sediment treatment groups

	Nominal IW Concentration	Measured IW Concentration	IW TU
Treatment A			
Fluoranthene	50	77	0.81
Treatment Group B (single contaminants at solubility limit)			
Anthracene	140	16	0.02
Benz(a)anthracene	44	9	0.79
2-Methylanthracene	26	30[a]	0.25
Benzo(b)fluoranthene	6	9[a]	0.55
Chrysene	18	6	0.45
3,6-Dimethylphenanthrene	48	48	0.64
Treatment Group C (treatment group B + treatment A [fluoranthene])			
Anthracene	140	58	0.08
Fluoranthene	50	80	0.84
Total (photoactive)	190	138	0.92
Total (all)	190	138	0.92
Benz(a)anthracene	44	52[a]	4.52
Fluoranthene	50	172	1.82
Total (photoactive)	94	224	6.34
Total (all)	94	224	6.34
2-Methylanthracene	26	55[a]	0.47
Fluoranthene	50	161	1.70
Total (photoactive)	76	216	2.17
Total (all)	76	216	2.17
Benzo(b)fluoranthene	6	14[a]	0.89
Fluoranthene	50	95	1.00
Total (photoactive)	56	109	1.89
Total (all)	56	109	1.89
Chrysene	18	7	0.52
Fluoranthene	50	72	0.76
Total (photoactive)	50	72	0.76
Total (all)	68	79	1.28
3,6-Dimethylphenanthrene	48	22	0.30
Fluoranthene	50	22	0.23
Total (photoactive)	50	22	0.23
Total (all)	98	44	0.53
Treatment Group D (mixture of six contaminants from treatment group B. Each at solubility limit)			
Anthracene	140	115	0.15
Benz(a)anthracene	44	80[a]	6.93
2-Methylanthracene	26	41[a]	0.35
Benzo(b)fluoranthene	6	20[a]	1.26
Chrysene	18	39[a]	2.91
3,6,Dimethylphenanthrene	48	202[a]	2.72
Total (photoactive)	216	256	8.69
Total (all)	282	497	14.32
(Mixture of six contaminants from treatment group B. Each contaminant at 25% of solubility limit)			
Anthracene	35	42	0.06
Benz(a)anthracene	11	15	1.31
2-Methylanthracene	6.5	10	0.08
Benzo(b)fluoranthene	1.5	4	0.21
Chrysene	4.5	7	0.49
3,6,Dimethylphenanthrene	12	59[a]	0.79
Total (photoactive)	54	71	1.66
Total (all)	70.5	137	2.94
Treatment Group E (treatment group D + treatment A. Each contaminant at solubility limit)[b]			
Anthracene	140	34	0.05
Benz(a)anthracene	44	14	1.24
2-Methylanthracene	26	7	0.06
Benzo(b)fluoranthene	6	4	0.22
Chrysene	18	6	0.48
3,6,Dimethylphenanthrene	48	42	0.57
Fluoranthene	50	34	0.36
Total (photoactive)	266	93	1.93
Total (all)	332	141	2.98
(Each contaminant at 25% of solubility limit)[b]			
Anthracene	35	30	0.04
Benz(a)anthracene	11	6	0.52
2-Methylanthracene	6.5	7	0.06
Benzo(b)fluoranthene	1.5	1	0.07
Chrysene	4.5	3	0.20
3,6,Dimethylphenanthrene	12	29	0.40
Fluoranthene	50	17	0.17
Total (photoactive)	104	61	0.86
Total (all)	120.5	93	1.46

Values are measured freely dissolved interstitial water (IW) concentrations (nmol/L) and calculated interstitial water toxic units (IW TU). Nominal IW concentrations were estimated by using the amount of contaminant amended to the sediment. Italicized contaminants are known (Swartz *et al.* 1997; Boese *et al.* 1998) or predicted by QSAR (Mekenyan *et al.* 1994) to exhibit enhanced toxicity when exposed to UV radiation

[a] Measured IW concentration exceeds empirically estimated IW solubility limit

[b] Fluoranthene excepted

bury in control sediment were again determined following the same protocol used for determining the initial test endpoints (ASTM 1996a). Cumulative results were reported (*e.g.*, number of amphipods unable to rebury after initial 10-day exposure = 10-day mortalities + number unable to rebury; number of phototoxic mortalities = initial 10-day mortalities + mortalities after 1-h UV exposure).

UV radiation and visible light were produced in the growth chamber by a combination of UV-A 340 and UV-B 313 fluorescent lamps (The Q-Panel Company, Cleveland, OH), and standard fluorescent lamps. To reduce UV-B intensities to levels that mimicked full sunlight, the petri dishes were covered with nylon window screening. An additional layer of cellulose acetate was added to remove any UV-C produced by the lamps. Previous experiments using this apparatus and lighting regime did not noticeably affect control *R. abronius* survival or reburial (Boese *et al.* 1997, 1998; Swartz *et al.* 1997). Light intensities ($\mu W/cm^2$) were measured from 250 to 800 nM at 1 nM intervals within the growth chamber using a spectroradiometer (Optronics model 752, Optronics Laboratories, Inc., Orlando, FL). Measurement and calibration procedures have been previously published (Boese *et al.* 1997).

Amphipod Tissue Samples

At t_0, a subsample of amphipods (n = 110) that were used to seed sediment exposure beakers was rinsed in distilled water to remove salt and adhering sediment particles, then blotted dry on laboratory tissue paper. This sample was weighted (± 0.1 mg) and subdivided into portions for lipid analysis (10 amphipods $\approx$ 0.04 g WW) and tissue residue analysis (100 amphipods $\approx$ 0.4 g WW). Amphipods sub-

sampled for lipids were reweighed, freeze-dried for 48 h (Flexi-Dry µP, FTS Systems, Stone Ridge, NY) and the sample weighted to determine the WW/DW ratio. Lipids were determined using a chloroform/methanol micro method (Gardner *et al.* 1985). Amphipods used for residue analysis were frozen (−80°C) until extraction.

At t_{10} surviving amphipods from four of the five replicates of each treatment were sieved from their reburial sediments, composited, rinsed with distilled water, and blotted dry. Amphipods (110) were counted from each of these samples and prepared for lipid and tissue residue analysis using the same procedures as used for the t_0 amphipod samples.

Chemical Analysis

Sediment from each of the abiotic chemistry replicates was centrifuged (3,200 *g*, 90 min, 15°C), and interstitial water (supernatant) was collected by aspiration (Ozretich and Schultz 1998). Total and bound IW PAH concentrations were determined using C18 solid-phase column fractionation (Ozretich *et al.* 1995). Each fraction was spiked with deuterated surrogate PAHs, gently extracted overnight with 10% (v/v) isooctane/hexane. The water layer was removed by aspiration with the remaining solvent layer reduced in volume using nitrogen gas. Deuterated phenanthrene was then added as a recovery standard and the extract quantified by GCMS (Hewlett Packard Model 5970, Palo Alto, CA).

PAH concentrations in tissues and sediments were determined using the method of Ozretich and Schroeder (1986). Acetonitrile extracts from the sonication of wet sediment (≈2 g) and from the sonication of the wet tissue of 100 amphipods (≈0.4 g) were cleaned using C18 solid-phase columns alone (sediments) or aminopropyl and C18 columns (tissues). The extracts were reduced in volume using rotoevaporation and the solvent exchanged to isooctane. Deuterated PAH surrogates were added prior to extraction, and a recovery standard was added to the final isooctane solution that was quantified by GCMS and corrected for loss of surrogate compounds.

Cadmium water samples from the positive controls were analyzed by flame atomic absorption spectrometry (Perkin Elmer Model 5100, Norwalk, CT). Results were quantified against standards prepared in acidified seawater at the salinity used in the toxicity tests.

Calculations

A toxic unit (TU) for an individual PAH was defined by Swartz *et al.* (1995) as:

$$TU_i = C_{IW}/\text{10-day } LC50_{IW} \qquad \text{(Eq. 1)}$$

where C_{IW} equals freely dissolved interstitial water concentration of an individual contaminant; and 10-day $LC50_{IW}$ is freely dissolved IW concentration needed to kill 50% of amphipods in a 10-day sediment toxicity test. $LC50_{IW}$ values for TU determinations were calculated using the relationship between measured $LC50_{IW}$ values for fluoranthene, acenapthene, and phenanthrene and their respective K_{ow} values (Swartz *et al.* 1995):

$$\text{Log 10-day } LC50_{IW}\ (\mu\text{mol/L}) = 5.92 - 1.33 \log K_{ow} \qquad \text{(Eq. 2)}$$

$$R^2 = 0.96,\ p < 0.001.$$

Nominal IW concentrations were estimated from sediment concentrations by:

$$C_{IW} = C_s/(K_{oc} \times f_{oc}) \qquad \text{(Eq. 3)}$$

where C_S is the sediment concentration (µmol/Kg DW); K_{oc} is the organic carbon-PAH/water partition coefficient (L/kg OC); and f_{oc} is the fraction organic carbon (kg OC/Kg DW). K_{oc} values were estimated from K_{ow} values using the relationship of DiToro (1985):

$$\text{Log } K_{oc} = 0.00028 + 0.983 \log K_{ow} \qquad \text{(Eq. 4)}$$

Bioconcentration factor (BCF) values were calculated from interstitial water concentrations by:

$$BCF = C_t/C_{IW} \qquad \text{(Eq. 5)}$$

where C_t is the tissue contaminant concentration (µmol/Kg DW); and C_{IW} is the interstitial water contaminant concentration (µmol/L). Biota-sediment bioaccumulation factor (BSAF) values were determined from sediment concentrations by:

$$BSAF = (C_t/L_t)/(C_s/f_{oc}) \qquad \text{(Eq. 6)}$$

where L_t is the tissue lipid concentration (Kg lipid/Kg tissue DW); and C_s is the sediment contaminant concentration (µmol/Kg DW).

Statistics

Statistical analyses were performed on a microcomputer using a spreadsheet (1-2-3®, Lotus Development Corp., Cambridge, MA) and a compatible statistical package (SigmaStat®, Jandel Scientific Software, San Rafael, CA). LC50 and EC50 values for individual treatments were calculated using the trimmed Spearman-Karber method (Hamilton *et al.* 1977). LC50 and EC50 values were also determined on a combined *R. abronius* data set using present results and those that have been previously published (Swartz *et al.* 1997; Boese *et al.* 1998). These latter calculations were by probit analysis (Finney 1971) using a PC SAS statistical package (SAS Institute Inc., Cary, NC).

Statistical comparisons of BCF and BSAF values across contaminants were done using ANOVA with significant differences between pairs of contaminants determined using pairwise multiple comparison procedures (Tukey test). Replicates (n = 9 for fluoranthene, n = 6 for all other contaminants) were obtained by calculating values singly for each contaminant within each of the treatment groups. Comparison between each contaminants (fluoranthene excluded) BCF and BSAF values determined at the compounds solubility limit and at 25% of that limit were examined using *t* tests (n = 4 and n = 2, respectively, for each compound).

Results

Quality Assurance/Quality Control

In the 10-day exposure tests, 147 of the 150 amphipods in negative control sediment and 148 of the 150 amphipods in

carrier control sediment survived, and all of these survivors were able to rebury in control sediment. This result meets QA/QC requirement for mortalities (≤10%) in amphipod toxicity tests (ASTM 1996a). Single negative and experimental control replicates were subsequently tested for phototoxicity. All 30 of the negative control and 29 of 30 of the carrier control amphipods that were exposed to 1 h of UV radiation were able to rebury in control sediment. To meet positive control QC limits at our laboratory, the Cd LC50 must fall within two standard deviations (0.19–2.00 mg Cd/L) of the combined means of all previous 4-day Cd toxicity tests that used *R. abronius.* The Cd LC50 of the present test was 1.56 mg Cd/L, a value that meets this criterium.

Uncontaminated sediment and interstitial water were spiked with SRM 1647c (NIST, Gaithersburg, MD) at approximately 1 to 10 µmol/Kg WW and 5 to 50 nmol/L respectively; uncontaminated pelagic shrimp homogenates were spiked with SRM2260 (NIST, Gaithersburg, MD) at approximately 2.5 µmol/Kg WW. Averaged ± SE recoveries for anthracene, fluoranthene, benzo(a)anthracene, chrysene, and benzo(b)fluoranthene were 79% ± 8%, 109% ± 8%, and 103% ± 1%, respectively for sediments, IWs, and tissues.

IW Concentrations and Toxic Units

Our success in achieving nominal IW concentrations varied with contaminant and, in some cases, measured IW concentrations exceeded empirical solubility limits (Table 2). This was especially apparent in the high concentration mixture treatments which did not contain fluoranthene (Table 2, treatment group D).

TUs (Table 2) were calculated directly from measured freely dissolved IW values using Equation 1 and Equation 2. TUs for contaminants with log K_{ow} values > 5.5 (chrysene, benzo(a)anthracene, and benzo(b)fluoranthene) were often greater than what we expected based on nominal sediment concentrations. This was especially apparent when empirical solubility limits were exceeded.

Tissue Residues

BCF values were highly variable for each contaminant (Table 3); however, with the exception of the generally twofold larger BCF values for fluoranthene (Tukey test, $p > 0.05$), BCF values were not significantly different among contaminants and across treatments (ANOVA, $p > 0.05$). There was no relationship between K_{ow} and BCF over the narrow K_{ow} range of the contaminants used in the present study.

BSAF values fell within two groupings, low values associated with contaminants that were added to sediments near their empirical aqueous solubility limits and higher BSAF values associated with the same contaminants added below their empirical solubility limits. These BSAF differences were significant (*t* test, $p < 0.05$). For fluoranthene the mean ± SE BSAF was relatively constant (10.18 ± 3.56), as fluoranthene was added to sediment at a nominally constant concentration that was always below its aqueous solubility limit. BSAF values for the contaminants other than fluoranthene that were added to sediment at concentrations designed to produce IW concentrations at 25% of aqueous saturation values ranged from 1.29 to 7.55 (Table 3, treatment groups D and E). The grand mean (control and treatment) lipid value (5.28% ± 0.28% on a dry weight basis) was used in these BSAF determinations as there was not a significant difference between control and treatment lipid values (*t* test, $p = 0.16$). Similarly, the grand mean OC value for all treatment sediments (2.57% ± 0.09%) was used in BSAF determinations.

Light Intensities

Light measurements within the growth chamber indicated that no light was present in the UV-C range (below 280 nm). Mean UV-B (280–320 nm), UV-A (321–400 nm), and visible light (401–700 nm) radiation intensities measured in the test were 97 ± 7 µW/cm^2 (mean ± SE), 227 ± 48 µW/cm^2, and 2,225 ± 89 µW/cm^2, respectively. These values roughly correspond to 76% of the UV-B, 9% of the UV-A, and 10% of the visible radiation present in full sunlight measured using the same instrument on a cloudless day (Oct. 3, 1996, 1 PM Pacific Daylight Time) at our location.

Initial and Phototoxic Responses

Initial (before UV) and photoactivated (after UV) mortality and reburial effects are compared in Table 4. All initial treatments were nontoxic as no differences were apparent between control and treatment initial (10-day) toxicities (Table 4) even though many of the treatments contained contaminants in excess of 1 TU (Table 2). In contrast to initial toxicities, all treatments, with the exception of treatment group B, were highly phototoxic. Initial (before UV) mortalities are similar to initial reburials (Table 4) as amphipods which survived the 10-day exposures were almost always able to rebury in control sediment. Post–UV exposure mortalities were often similar to before-UV 10-day mortalities (Table 4) as even amphipods that were severely effected by UV-induced toxicity were often initially able to survive the 1-h exposure to UV radiation. However, if these surviving amphipods had bioaccumulated an effective internal dose of a phototoxic contaminant, they exhibited similar symptoms, which included slowed pleopod movements with relaxed or recurved bodies in contrast to the flexed bodies of unaffected amphipods. Amphipods that displayed these symptoms were unable to rebury in sediment and, as has been observed in previous experiments (Boese *et al.* 1997, 1998), die within a few hours following the UV exposure. Thus amphipods which could not rebury following the 1-h UV exposure should be considered as mortalities.

Critical Body Residue (CBR)

As initial mortalities were not observed in the present study, a direct measure of the CBR for *R. abronius* was not possible.

Table 3. Amphipod tissue concentrations (Ct, µmol/Kg DW), interstitial water bioconcentration factors (IW BCF), and biota sediment accumulation factors (BSAF)

	Ct	IW BCF	BSAF
Treatment A			
Fluoranthene	2,329	30,275	13.34
Treatment Group B (Single contaminants at solubility limit)			
Anthracene	436	26,786	1.55
Benz(a)anthracene	174	19,217	0.56
2-Methylanthracene	342	11,596	2.29
Benzo(b)fluoranthene	43	4,888	0.62
Chrysene	9	1,560	0.07
3,6-Dimethylphenanthrene	1,207	25,289	1.84
Treatment Group C (treatment group B + treatment A)			
Anthracene	596	10,294	2.22
Fluoranthene	3,530	44,017	15.19
Benz(a)anthracene	310	5,978	1.09
Fluoranthene	3,186	18,470	12.31
2-Methylanthracene	491	8,936	3.28
Fluoranthene	3,075	19,102	12.35
Benzo(b)fluoranthene	34	2,357	0.59
Fluoranthene	3,194	33,599	13.25
Chrysene	28	3,934	0.19
Fluoranthene	2,270	31,618	9.05
3,6-Dimethylphenanthrene	607	27,191	1.25
Fluoranthene	1,100	50,484	4.98
Treatment Group D (mixture of six contaminants from treatment group B. Each at solubility limit)			
Anthracene	376	3,267	1.24
Benz(a)anthracene	225	2,832	0.82
2-Methylanthracene	182	4,410	1.08
Benzo(b)fluoranthene	34	1,657	0.46
Chrysene	44	1,141	0.28
3,6,Dimethylphenanthrene	1,720	8,510	2.37
(Mixture of six contaminants from treatment group B. Each at 25% of solubility limit)			
Anthracene	255	6,057	4.32
Benz(a)anthracene	181	12,092	2.78
2-Methylanthracene	167	16,842	4.46
Benzo(b)fluoranthene	22	6,280	1.70
Chrysene	69	10,495	1.57
3,6,Dimethylphenanthrene	1,250	21,255	7.55
Treatment Group E (treatment group D + treatment A. Each at solubility limit)[a]			
Anthracene	323	9,282	1.20
Benz(a)anthracene	130	9,116	0.53
2-Methylanthracene	142	20,209	0.88
Benzo(b)fluoranthene	17	4,933	0.29
Chrysene	28	4,366	0.18
3,6,Dimethylphenanthrene	1,022	24,109	1.44
Fluoranthene	1,660	48,587	6.66
(Each contaminant at 25% of solubility limit)[a]			
Anthracene	189	6,298	3.27
Benz(a)anthracene	152	25,465	2.53
2-Methylanthracene	140	21,399	3.45
Benzo(b)fluoranthene	19	16,772	1.57
Chrysene	56	21,080	1.29
3,6,Dimethylphenanthrene	1,013	34,440	6.27
Fluoranthene	513	30,918	8.62

Italicized contaminants are known (Swartz *et al.* 1997; Boese *et al.* 1998) or predicted by QSAR (Mekenyan *et al.* 1994) to exhibit enhanced toxicity when exposed to UV radiation. Ct and IW BCF may be converted to their tissue WW equivalents by multiplying DW values by the DW/WW ratio for *R. abronius* (0.197)

[a] Fluoranthene excepted

However, the CBR was estimated using the linear relationship between total tissue residues and interstitial water contaminant concentrations (Figure 1). Using this relationship and IW LC50 values for four compounds (Boese *et al.* 1998) with K_{ow} values similar to the contaminants used in the present study, we estimated the CBR for *R. abronius* to range from 2,700 to 11,600 µmol/Kg DW (Figure 1) which corresponds to 530 to 2,270 µmol/Kg WW. The highest tissue concentrations (Table 4) were in the mixture treatments (treatment groups C, D, and E) where tissue residues ranged from approximately 1,700 to 4,100 µmol/Kg tissue DW (330 to 800 µmol/Kg tissue WW). Surprisingly, several of the tissue residue values measured in the present study were within this estimated CBR range, but did not result in any initial (10-day) mortalities.

Discussion

Initial 10-Day Toxicities

In a previous study conducted at our laboratory (Swartz *et al.* 1997), the toxicity of mixtures of contaminants, each of which was capable of causing amphipod mortalities in single compound exposure experiments, was examined. Although the results of that study indicated that TUs were slightly less than additive (mean ΣTU LC50 = 1.55), the authors concluded that the assumption of additivity allows a reasonably accurate and environmentally protective estimate of the toxicity of PAH mixtures (Swartz *et al.* 1997). In contrast, no toxicity was observed in any of the mixture treatments used in the present study even though almost all of the ΣTUs exceeded 1.0 and several exceeded 2.0 (Table 2, treatments C–E). This result suggests that the 10-day amphipod toxicities of highly lipophilic sediment contaminants are not additive and that extrapolation of the existing ΣPAH model to include contaminants that are nontoxic in single-contaminant 10-day tests may seriously overestimate a sediment's toxicity potential.

The ΣPAH model's predictive failure may be due to several factors. First, TUs were estimated from the relationship between amphipod K_{ow} and IW LC50s (Equation 2) that were determined in 10-day sediment toxicity tests. The original relationship (Swartz *et al.* 1995) utilized only three PAHs (fluoranthene, acenaphthene, and phenanthrene), with fluoran-

Table 4. Amphipod reburial responses, sum of tissue concentrations (ΣCt), and sum of interstitial water toxic units (ΣTU)

Treatment	ΣTU (All)	ΣCt (All)	ΣCt (Photox)	% Survival Before UV[a]	% Survival After UV[b]	% Reburial Before UV[a]	% Reburial After UV[b]
Treatment A							
Fluoranthene (Flu)	0.81	2,329	2,329	96	97	96	0
Treatment Group B							
Anthracene	0.02	436	436	98	100	98	100
Benz(a)anthracene	0.79	174	174	98	97	98	97
2-Methylanthracene	0.25	342	342	98	97	98	97
Benzo(b)fluoranthene	0.55	43	43	96	100	96	97
Chrysene	0.45	9	0	96	100	96	97
3,6-Dimethylphenanthrene	0.64	1,207	0	97	99	97	97
Treatment Group C							
Anthracene + Flu	0.92	4,126	4,126	96	33	96	0
Benz(a)anthracene + Flu	6.34	3,496	3,496	97	47	97	0
2-Methylanthracene + Flu	2.16	3,566	3,566	98	0	97	0
Benzo(b)fluoranthene + Flu	1.89	3,227	3,227	98	97	98	0
Chrysene + *Flu*	1.28	2,297	2,270	98	93	97	0
3,6-Dimethylphenanthrene + *Flu*	0.53	1,707	1,100	99	100	100	70
Treatment Group D							
Mixture at solubility limit	14.32	2,581	817	96	50	96	0
Mixture at 25% solubility limit	2.95	1,944	625	95	77	95	0
Treatment Group E							
Mixture at solubility limit + Flu	2.97	3,321	2,272	93	13	91	0
Mixture at 25% solubility limit + Flu	1.45	2,082	1,014	100	17	100	0
Controls							
Experimental	>DL	>DL	>DL	98	100	98	97
Negative	>DL	>DL	>DL	99	99	100	100

Ct values µmol/Kg DW (DW to WW conversion factor 0.197). Reburial response values are the percent of initially exposed amphipods that were able to rebury in control sediment following an initial 10-day exposure to PAH-contaminated sediments (before UV) followed by a 1-h UV exposure (after UV) of the survivors of the 10-day test. Italicized contaminants are known (Swartz *et al.* 1997; Boese *et al.* 1998) or predicted by QSAR (Mekenyan *et al.* 1994) to exhibit enhanced toxicity when exposed to UV radiation

[a] Percentage based on 150 total amphipods in five replicates that were exposed at test initiation (t_0) for 10 days

[b] Percentage based on the single replicate (30 amphipods) exposed to 1 h of UV at the end of the 10-day test (t_{10})

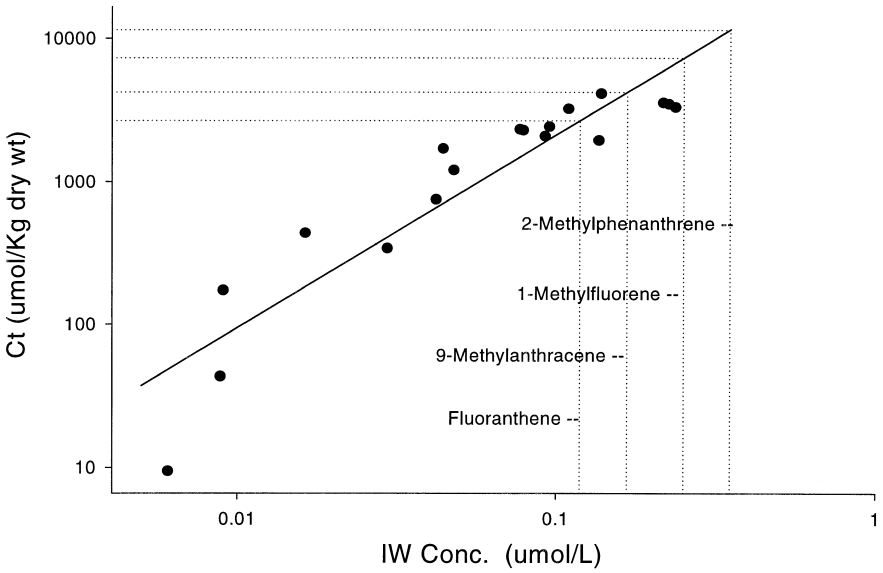

Fig. 1. Relationship between the sum of freely dissolved interstitial water (IW) contaminants and the sum of tissue contaminant concentrations (Ct) from treatment groups A–E (solid circles). Values from the solubility limit treatment (treatment group D) were excluded. Solid line is the least-squares linear regression for these data: logCt = 4.68 + 1.35(logIW), $R^2 = 0.85$, $p < 0.001$). Dotted lines to X axis are the IW LC50 values four sediment contaminants: 9-methylanthracene, 1-methylfluorene, 2-methyphenanthrene from Boese *et al.* (1998) and fluoranthene from Swartz *et al.* (1995). Dotted lines to Y axis are the estimated tissue residues (CBR estimates) associated with these LC50

thene having the largest log K_{ow} (5.22). Additional LC50 values for naphthalene, 2,6-dimenthylnapthalene, 1-methylfluorene, 2-methyphenanthrene, and 9-methylanthracene (Boese *et al.* 1998) have recently been added to this regression with no significant changes noted in either the slope or intercept (R. J. Ozretich, personal communication). However, as none of these compounds have K_{ow} values in excess of fluoranthene, we have no data to support the assumption that the relationship is linear

for compounds with log $K_{ow} > 5.22$. A similar relationship between K_{ow} and water-only 96-h LC50s for fish species was shown to be bilinear with a noticeable reduction in the rate of increase in toxicity for compounds with log $K_{ow} > 4$ (Veith *et al.* 1983). If similar reductions in toxicity occurred in the present study, extrapolating the existing IW toxicity relationship would overestimate the toxicity contribution of high K_{ow} contaminants.

Second, an evaluation of Veith's data (Veith *et al.* 1983) by McCarty *et al.* (1992) suggests that the critical tissue residue or CBR (*i.e.*, the tissue residue that has a toxic effect) of all neutral narcotics are similar and therefore additive. According to this reasoning, compounds with larger K_{ow} values are not inherently more toxic, they simply attain the CBR at lower aqueous concentrations due to their larger BCF values. Although there is a well-established and linear relationship between BCF and K_{ow} (Veith *et al.* 1979; Barron 1990), this relationship was not observed in the present study, possibly due to the narrow range of contaminant K_{ow} values (Table 1). In addition, Oliver and Niimi (1985) observed that as molecular weight increases and log K_{ow} values exceed five to six the correlation between BCF and K_{ow} is poor, with many contaminants exhibiting BCF values lower than expected. Although a variety of causes for this loss of linearity at high K_{ow}s have been proposed (Gobas *et al.* 1989; Hawker and Connel 1985; Spacie and Hamelink 1982; Tulp and Hutzinger 1978), a likely cause for this in the present study was that a 10-day exposure was insufficient for tissue residues to reach their full potential for bioaccumulation. To test this hypothesis, additional experiments need to be conducted with these contaminants using longer duration experiments such as the 28-day chronic amphipod toxicity test (DeWitt *et al.* 1992).

Tissue Residues

The most likely explanation for why BSAF values fell in two groupings is that all of the contaminants (fluoranthene excepted) were amended to exposure sediments at two concentrations, the largest of which was an attempt to attain IW concentrations that were at or near the aqueous saturation limit for each contaminant. If the most important uptake route into amphipods was via IW, contaminants amended to sediments in excess of the amount needed to saturate IW, would tend to reduce the measured BSAF. IW BCF values support this interpretation as they did not fall into statistically distinguishable high and low groupings. Thus, the more reliable BSAF values are for fluoranthene and for the other contaminants that were amended to sediment at concentrations designed to produce IW concentrations at 25% of their estimated saturation values (Table 3).

These more reliable BSAF values were five- to tenfold larger than expected. Although BSAF values in excess of 2.0 are often observed in laboratory spiked sediment for metabolically refractory compounds such as PCBs (Boese *et al.* 1995; Tracy and Hansen 1996), high BSAF values have not been observed for PAHs using a wide variety of compounds and species (Tracy and Hansen 1996). Although there are fewer BSAF values for amphipods, reported values for fluoranthene-, phenanthrene-, and pyrene-spiked sediments have generally been found to be < 1 in freshwater amphipods (Boese and Lee 1992; Driscoll *et al.* 1997a). However, when using the estuarine amphipod *Leptocheirus plumulosus*, Driscoll *et al.* (1996) reported BSAF values that ranged from 1.36–1.87 following 27 days of exposure to [^{14}C]-fluoranthene-spiked sediment. Although these latter values are somewhat higher than the values reported for freshwater amphipods, they are still an order of magnitude lower than those observed in the present study using *R. abronius*. In contrast, Fuji (1997) found larger BSAF values for [^{14}C]-phenanthrene in the estuarine amphipods *Eohaustorius estuarius* (max. BSAF = 3.29), *Leptocheirus plumulosus* (max. BSAF = 4.11), and *Grandidierella japonica* (max. BSAF = 2.51). Although these values are still lower than those observed for fluoranthene in the present study, they were determined after only 3 days of exposure, which may have been of insufficient duration for phenanthrene to attain steady-state tissue residues.

A partial explanation for these high BSAF values is that phoxocephalid amphipods, like *R. abronius*, are predaceous omnivores (Oakden 1984) who consume prey and detritus that contains higher OC and associated contaminants than the aggregate sediment. Gut enrichment of this kind has been used to explain the larger than expected BSAF in selective deposit-feeding clams (Boese *et al.* 1996) and as a mechanism for biomagnification in fish (Gobas *et al.* 1993). High internal (gut) exposure to PAHs and the possibility that *R. abronius* may have a relatively low ability to metabolize these contaminants would tend to increase BSAF values. Regardless of the cause, phoxocephalid amphipods appear to have a large bioaccumulation potential for PAHs, which may in part explain why this taxa is one of the first to decline in response to a field pollution gradient (Swartz *et al.* 1982, 1986).

The CBR estimate for *R. abronius* (2,700 to 11,600 µmol/Kg DW = 530 to 2,270 µmol/Kg WW) is considerably lower than the CBR values reported by McCarty *et al.* (1992) for fish species (2,200–8,300 µmol/Kg WW). McCarty points out a possible relationship between CBR and tissue lipids with CBR increasing with lipid content. In fish with the lowest lipid content (3% wet weight basis) McCarty *et al.* (1993) noted that the mean CBR was reduced to 2,600 (2,200–3,100) µmol/kg WW. As the amphipod used in the present study had a lipid content of only 1% on a wet-weight basis (5% dry-weight basis), an even lower CBR is possible. The higher CBR values reported for freshwater amphipods (Landrum *et al.* 1994; Driscoll *et al.* 1997a), may also partially be explained by this effect as one of the amphipods (*Diporeia* sp.) used in those studies had a lipid value of 21% on a wet-weight basis. The other amphipod species used in that study, *Hyallella azteca*, was closer in lipid content (7% dry-weight basis) to *R. abronius*, but did not bioaccumulate more than 1,000 µmol/kg tissue WW of fluoranthene, which, as in the present experiment, was not sufficient to cause mortality (Driscoll *et al.* 1997a). In an additional study using *H. azteca* exposed to water-only exposures, the CBR for fluoranthene was estimated to be 560 and 360 µmol/Kg WW in two separate experiments (Driscoll *et al.* 1997b). CBR values reported for *Daphnia magna* (Pawllsz and Peters 1993) with similar lipid content to that of *R. abronius*, was 3,100 (1,100–5,100 µmol/Kg WW), which overlaps a portion of the present CBR estimates. In addition, an estimate of the CBR for *L. plumulosus*, which is similar in lipid content to *R. abronius*, was 694 µM/Kg WW (Driscoll *et al.* 1996). All these results are consistent with the hypothesis that CBR is proportional to lipid content. As lipid content increases, the proportion of energy storage lipids to structural lipids would

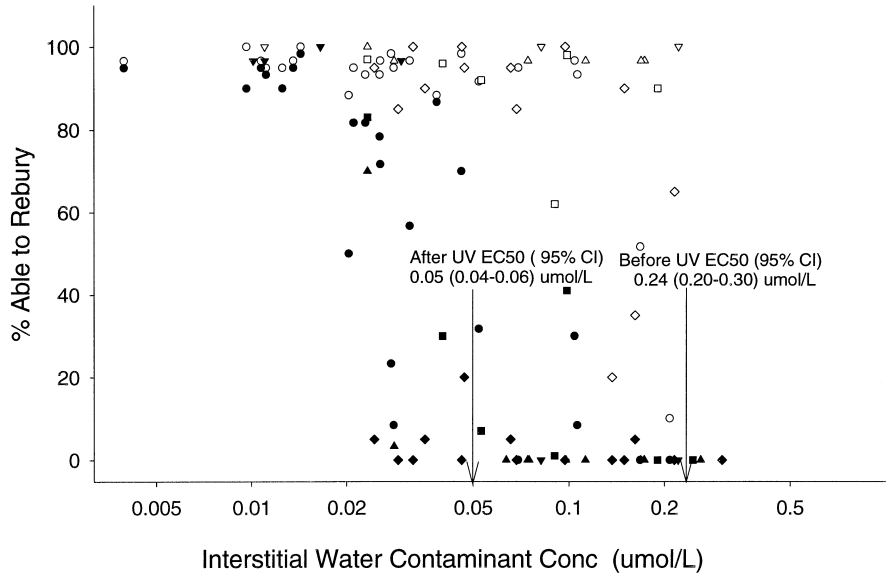

Fig. 2. Combined data comparison between percent of amphipods able to rebury immediately following an initial 10-day exposure to sediments spiked with PAHs (open symbols) and after a subsequent 1-h UV exposure (solid symbols), verses the sum of freely dissolved phototoxic PAHs (open symbols). Reburial EC50 (95% CI) were derived by probit analysis using data from the present experiment and from previously published data (Swartz *et al.* 1997; Boese *et al.* 1998). Symbols are as follows: ○, ● = single-contaminant exposures from Boese *et al.* (1998); ◇, ◆ = single-contaminant exposures; □, ■ = mixture exposures from Swartz *et al.* (1997); ∇, ▼ = single-contaminant exposures; Δ, ▲ = mixture exposures from the present study

tend to increase, suggesting that neutral narcotics associated with energy storage lipids may not be as toxic as those associated with structural lipids.

An unexplained and disconcerting result of the present study is that although several tissue residue values are larger than these amphipod CBRs and were within the CBR range estimated for *R. abronius* (Figure 1), no initial (10-day) toxicity was evident.

Phototoxicity

Although the treatments used in the present study were not initially toxic, all treatments with the exception of the single-contaminant exposures in treatment group B, became toxic when amphipods were removed from sediment and exposed to UV radiation for 1 h (Table 4). The lack of phototoxicity in treatment group B was most likely due to the low tissue residues associated with this treatment in comparison to treatment groups in which a phototoxic response was observed (Table 4).

The all-or-nothing phototoxic response observed in the present experiment is difficult to interpret, but when combined with the results of other phototoxicity experiments, a clearer picture emerges. Figure 2, which is limited to phototoxic contaminants, combines the results of the present experiment with those of two previously published data sets (Swartz *et al.* 1997; Boese *et al.* 1998), which used *R. abronius* exposed under similar conditions. For phototoxic contaminants, the reburial IW EC50 (95% CI) following UV exposure (determined by probit analysis) was 0.050 (0.040–0.063) µmol/L. This value is 20% of the initial (before UV) reburial IW EC50 value of 0.243 (0.206–0.302) µmol/L and statistically different based on nonoverlapping 95% CI intervals. Although there is considerable scatter in the data, the toxicity of mixtures of phototoxic contaminants appears to be consistent with the toxicity of single phototoxic contaminants when expressed on a total µmol/L basis. This result suggests that the toxicity of phototoxic sediment contaminants are roughly additive on a molar basis. However, the conclusion is tentative as it ignores numerous sources of uncertainty. First phototoxicity is proportional to the UV intensity and duration (Ankley *et al.* 1995). Although exposure duration was identical in all three of these studies, UV intensities varied twofold. Second, photoactive compounds vary in their phototoxicity potential. For example, fluoranthene has been shown to be only 25% as toxic as anthracene and pyrene at equal tissue concentrations (Ankley *et al.* 1995). Thirdly, for a contaminant to be phototoxic, it must be bioaccumulated by the organism in sufficient quantity to evoke an observable phototoxic response (Ankley *et al.* 1995) and bioaccumulation potential varies with K_{ow} and the length of exposure (ASTM 1996b). This last uncertainty should be reduced if tissue residues rather than IW concentrations were used.

The unanswered question of this and other research into the phototoxicity of contaminated sediments is whether phototoxicity is of ecological significance or merely an interesting laboratory artifact. Although the effects of UV-induced on *R. abronius* is dramatic, phototoxicity may have little ecological significance in this species. In nature, infaunal amphipods generally do not emerge in daylight and are therefore unlikely to experience phototoxic effects. In a comparative study (Boese *et al.* 1997) showed that phototoxicity to fluoranthene was greatly reduced in infaunal crustaceans that are naturally exposed to sunlight. It is likely that infaunal organisms that do emerge into full sunlight have evolved means to protect themselves from the direct effects of UV radiation (*e.g.*, pigmentation) that would also tend to protect them from photoinduced toxicity. In addition, the method used to evaluate phototoxicity in the present research has little relation to how infaunal species would be exposed to sunlight in nature. Regardless of its ecological relevance, the phototoxicity responses observed in the present study indicated that test amphipods had bioaccumulated significant and potentially lethal amounts of sediment PAH contaminants. This information would have been missed if only a standard 10-day amphipod toxicity test had been performed.

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